The clusters generated were not large enough to settle out of solution. However, they could be separated by centrifugation at relatively low speeds (10,000 RPM for 10 min), as compared with the unlinked particles (30,000 RPM for 2-3 hours).

The decrease in fluorescence upon hybridization was determined by integration of the fluorescence signal (320 nm excitation wavelength) from 475 nm to 625 nm of 4 pairs of samples. Each pair was prepared in the following manner. A solution of of 3' propylthiol-terminated DNA-modified particles (30 μL, optical density at 530 nm = 0.224) was combined with a solution of 5' hexylthiol-terminated DNA-modified QDs (30 μL, optical density at 530 nm = 0.206) in an Eppendorf centrifuge tube, and then diluted with 140 μL of PBS. The mixture was then split into two equal portions, and complementary "linker" DNA (3 μL, 30 pmol) was added to one, while non-complementary "linker" DNA (5'-CTACTTAGATCCGAGTGCCCACAT-3', SEQ ID NO: 49) (3 μL, 30 pmol) was added to the other. All eight of the samples were then frozen in a dry ice/acetone bath (-78°C), after which they were removed from the bath and allowed to warm slowly to room temperature. To estimate the change in fluorescence efficiency upon hybridization, the fluorescence intensities of the "target" (complementary "linker") samples were adjusted to account for the difference in absorbance at 320 nm from the corresponding control samples, which contained non-complementary "linker".

The results showed that hybridization of QD/QD assemblies was accompanied by a decrease in integrated fluorescence intensity by an average of 26.4 ± 6.1%, and a ~2 nm red shift of the emission maximum, presumably due to cooperative effects between QDs, Figure 27A. Interestingly, Bawendi, *et al.* noticed a similar, albeit slightly larger, red shift when comparing the fluorescence of close-packed QDs and widely separated dots isolated in a frozen matrix (Murray et al., *Science* **1995**, *270*, 1335). These changes in the fluorescence spectra may be an indication of excimer formation between QDs, but the exact nature of such a complex is still largely a matter of speculation. As expected, no aggregation was observed

when the "linker" was missing or not complementary, or when either one of the two types of particles was absent.

The "melting" behavior of the DNA was monitored by observing the UV-Vis spectra of the aggregates as a function of temperature. For this "melting" analysis, the precipitate containing the QD/QD assemblies was centrifuged at 10,000 rpm for 10 minutes, washed with 7 μL of PBS, recentrifuged, and suspended in 0.7 mL of PBS. The UV/Visible spectroscopic signature of the assemblies was recorded at two degree intervals as the temperature was increased from 25°C to 75°C, with a holding time of 1 minute prior to each measurement. The mixture was stirred at a rate of 500 rpm to ensure homogeneity throughout the experiment. Temperature vs extinction profiles were then compiled from the extinction at 600 nm. The first derivative of these profiles was used to determine the "melting" temperatures.

The results, Figure 27B (T_m = 57°C), demonstrated conclusively that DNA had been immobilized on the QD surfaces and that hybridization was responsible for the assembly process. The transition also was extremely sharp when compared with DNA alone (FWHM of the respective first derivatives: 4°C vs 9°C), which is consistent with the formation of an aggregate structure with multiple DNA links per particle. An increase in extinction was observed upon denaturation, most likely because of a screening effect whereby particles in the interiors of the assemblies are prevented from absorbing light by the surrounding QDs.

D. Preparation Of QD/Gold Assemblies

With DNA-functionalized QDs in hand, the assembly of hybrid assemblies made from multiple types of nanoparticle building blocks became feasible. To prepare these hybrid assemblies, a solution of ~17 nM 3'-hexylthiol-modified 13 nm gold nanoparticles (30 μL, ~5 fmol; prepared as described in Example 3) was mixed with a solution of 5'-hexylthiol-terminated DNA-modified QDs (15 μL, optical density at 530 nm = 0.206) in an Eppendorf centrifuge tube. "Linker" DNA (5 μL, 50 pmol) was added, and the mixture cooled to -78°C, and then allowed to warm slowly to room temperature, generating a reddish-purple precipitate. No aggregation behavior was observed unless both types of particles and

a complementary target were present. After centrifugation (1 min at 3,000 rpm) and removal of the supernatant, the precipitate was washed with 100 μL of PBS and recentrifuged.

For "melting" analysis, the washed precipitate was suspended in 0.7 mL of PBS. UV-Vis spectroscopy was used to follow the changes in the surface plasmon resonance of the gold nanoparticles, so temperature vs. extinction profiles were compiled at 525 nm. Using the surface plasmon resonance of the gold nanoparticles provides a much more sensitive probe with which to monitor hybridization than does the UV-Vis spectroscopic signature of the QDs alone. Therefore, a "melting" experiment can be performed on a much smaller sample (~10% of the QD solution is needed), although the intensity of the plasmon band obscures the UV/Vis signal from the QDs. Similar to the pure QD system described above, a sharp (FWHM of the first derivative = 4.5°C) melting transition occurred at 58°C (see Figure 27D).

High resolution TEM images of these assemblies showed a network of gold nanoparticles interconnected by multiple QDs, Figure 27C. The QDs, which have a much lower contrast in the TEM image than gold nanoparticles, can be identified by their lattice fringes. They are just barely resolvable with the high resolution TEM, but clearly indicate the periodic structure of these composite assemblies and the role that DNA plays in forming them.

E. Summary

The results described in this example definitively establish that the immobilization of DNA onto QD surfaces has been achieved and that these particles can now be used in combination with DNA under hybridization conditions. Using DNA-functionalized QDs, the first DNA-directed formation of QD and mixed gold/QD nanoparticle structures has been demonstrated. The successful modification of semiconductor QDs with DNA has significant implications for materials research, and the door is now open for more extensive inquiries into the luminescent, electronic, and chemical properties of these unique building blocks as they are incorporated into new and functional *multi-component* nanostructures and nanoscale materials.